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(71) Applicants *(for all designated States except US)*: SUOMEN PUNAINEN RISTI VERIPALVELU [FI/FI]; Kivihaantie 7, FIN–00310 Helsinki (FI). TÖLÖ, Hannele [FI/FI]; Ulvilantie 16 C, FIN–00350 Helsinki (FI). PARKKINEN, Jaakko [FI/FI]; Liinasaarentie 21 B, FIN–02160 Espoo (FI).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: KAUPPINEN, Hanna–Leena [FI/FI]; Jalavatie 5 A 8, FIN–00270 Helsinki (FI). ALM, Gunnar [SE/SE]; Arkitektvägen 44, S–740 20 Vänge (SE).

(74) Agent: SEPPO LAINE OY; Itämerenkatu 3 B, FIN–00180 Helsinki (FI).

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(54) Title: ALPHA–INTERFERON MANUFACTURING PROCESS USING IMMUNOADSORPTION AND VIRUS REMOVAL FILTRATION

(57) Abstract

The present invention concerns a method of manufacturing α–interferon product from crude interferon preparations obtained from human or animal cell culture or from transgenic animals. The method comprises contacting a solvent/detergent treated composition with at least two monoclonal mouse IgG antibodies having complementary subtype specificities in an immunoadsorption step. The α–interferon subtypes bound by the monoclonal antibodies are eluted and the eluate is purified and filtered on a virus removal filter. The method according to the invention effectively removes all types of impurities present in crude interferon products, and gives rise to a highly purified and virus–safe multicomponent interferon which contains major α–interferon subtypes.

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ALPHA-INTERFERON MANUFACTURING PROCESS USING IMMUNOADSORPTION AND VIRUS REMOVAL FILTRATION

Background of the Invention

Field of the Invention

The present invention relates to the manufacture of α-interferon drugs from human or animal cell culture or from transgenic animals. In particular, the invention relates to the manufacture of a highly purified and virus-safe α-interferon solution, which contains major natural α-interferon subtypes.

Description of Related Art

α-Interferons (IFN-α) are important drugs with antiproliferative, antiviral and immunomodulatory effects. Human leukocytes are known to produce several IFN-α subtypes in culture when induced by Sendai virus (Nyman et al., Biochem. J. 329, 295-302, 1998). Human lymphoblastoid cells produce similarly several IFN-α subtypes after Sendai virus induction (Zoon et al., J. Biol. Chem. 267, 15210-15216, 1992). Both leukocyte and lymphoblastoid IFN-α (hereinafter called "multicomponent α-interferon") are used as drugs in the treatment of neoplastic and viral diseases.

IFN-a drugs are also produced by recombinant DNA technology in bacteria.The recombinant bacterial products contain a single IFN-a subtype, which differs structurally from the corresponding natural subtypes. Recombinant bacterial products may induce antibodies against IFN-a in patients, which may result in the disappearance of the therapeutic response. Multicomponent IFN-a products have restored the therapeutic response in these patients, and multicomponent products may have also other therapeutic benefits in comparison with recombinant bacterial products (Farrell, Hepatology 26, 96S-100S, 1997). The therapeutic benefits of multicomponent IFN-a are evidently based on the native structure of the IFN-a_subtypes in these products. It is also possible to produce native IFN-a subtypes with recombinant DNA technology in cultured animal cells or transgenic animals, which, unlike the recombinant bacterial products, would contain the correct structure even with respect to posttranslational modifications.

An important goal in the purification of IFN-α from human or animal cell culture is to

include all major natural subtypes in the finished product. This must take place in a reproducible way in order to have a constant subtype composition in the product, which is a prerequisite for the constant quality of the drug. Further, it is equally important in the purification of IFN-α from human or animal cell culture or from transgenic animals that viruses present in the starting materials are inactivated and removed. Leukocytes, cell lines, serum and its fractions and animals used in the production of natural IFN-α subtypes may harbour viruses and other infectious agents of diverse physicochemical properties, which cannot be effectively inactivated by a single virus inactivation treatment.

Blood-borne viruses potentially present in serum fractions and leukocytes include enveloped viruses, such as HI-viruses, hepatitis C and B viruses, and also non-enveloped viruses resistant to many physicochemical treatments, such as parvovirus B19. Lymphoblastoid cell lines may harbour e.g. retroviruses.

Several manufacturing processes have been developed, which use human leukocyte culture as the starting material to obtain a multicomponent IFN-α drug.

US Patent No. 5,391,713 describes a purification process for leukocyte interferon comprising an immunoaffinity chromatography step, in which a polyclonal goat antibody against IFN-α is used. The purification process comprises precipitation steps with thiocyanate solution and aqueous ethanol, but no other specific virus inactivation or removal steps. The use of polyclonal antibodies in the purification may be problematic since the specificity of the antibodies obtained in different immunization times may change and result in changes in the subtype composition and impurity profile of the drug purified with different antibody batches.

US Patent No. 5,503,828 concerns a purification process of leukocyte interferon comprising immunoaffinity chromatography with a monoclonal antibody NK2. The use of this monoclonal antibody results in a composition, which consists of at least 50 % of the IFN-α subtypes α2 and α8, and additional subtypes from the group α4, α7, α10, α16, α17 and α21. However, during the purification process, at least subtypes IFN-α1, IFN-α21 and IFN-α14 are lost. Of these subtypes, IFN-α1 is the most abundant natural subtype (Nyman et al., Biochem. J. 329, 295-302, 1998). The purification process contains low pH treatment for at least 2 days but no other, specific virus inactivation or removal steps.

It may be possible to obtain all major IFN-α subtypes by immunoaffinity chromatography

with a polyclonal antibody, but in practice it appears impossible to obtain polyclonal antibodies which would bind only IFN-α and not other closely related cytokines. Further, it is not possible to a have constant supply of polyclonal antibodies with identical binding specificity. Therefore, the use of polyclonal antibodies in the purification does not ensure constant subtype composition and impurity profile of the product in a long term. The use of a monoclonal antibody in the immunoaffinity chromatography allows for constant binding specificity in a long term production. However, at least in case of the NK2 antibody, the use of only one monoclonal antibody resulted in the loss of major IFN-α subtypes from the product.

The current methods for the purification and manufacture of leukocyte interferon do not comprise several specific virus inactivation or removal steps with different mode of action and they do not allow for inactivation of viruses resistant to e.g. low pH or aqueous ethanol and thiocyanate.

Summary of the Invention

It is an object of the present invention to eliminate the problems of the prior art and to provide a method for the manufacture of highly purified IFN-α solution from cell cultures or transgenic animals. In particular, the invention aims at providing a novel method for producing a higly purified, virus-safe multicomponent IFN-α solution from crude interferon preparations.

This and other objects, together with the advantages thereof over known processes, which shall become apparent from specification which follows, are accomplished by the invention as hereinafter described and claimed.

According to the present invention there is provided a manufacturing process for multi-component IFN-α, comprising multiple virus elimination steps. The present invention is based on the finding that by subjecting an intermediate proteineous composition of an IFN-α isolation process to immunoadsorption chromatography with at least two monoclonal antibodies, which have complementary subtype specificities, it is possible not only to separate reproducibly all major IFN-α subtypes but also effectively to remove the most persistant infectious agents from IFN-α, including non-enveloped viruses and physico-chemically resistant infectious agents, such as those causing transmissible spongiform encephalopathies ("prions").

More specifically, the present invention is characterized by what is stated in the characterizing part of claim 1.

The invention provides considerable advantages. Thus, in short, by combining immunoadsorption chromatography with a preceding solvent/detergent treatment, and with a subsequent treatment at low pH and filtration with a virus removal filter, it is possible to achieve four efficacious virus elimination steps, of which at least two eliminate of all types of viruses and infectious agents potentially present in the cell cultures. The present process has the capacity to purify extensively all major natural IFN-α subtypes with a good yield and continuously give rise to a product with a constant subtype composition.

Next, the invention will be examined more closely with the aid of the following detailed description and with reference to a number of specific embodiments.

Brief Description of the Drawings

In the attached drawings,
Figure 1 shows in the form of a flow diagram of a preferred embodiment of the present process for producing highly purified, virus-safe IFN-α drug containing all major IFN-α subtypes; and
Figure 2 shows a typical reverse phase-HPLC profile of the purified leukocyte IFN-α drug substance.

Detailed Description of the Invention

The present invention describes a method for producing highly purified α–interferon product. Starting from leukocytes, the process generally comprises the steps of removing the cells from a leukocyte culture, concentrating the crude interferon thus obtained, filtering and subjecting the crude interferon concentrate to solvent/detergent treatment (in the following also abbreviated S/D treatment), binding IFN-α from the S/D treated crude interferon concentrate with at least one monoclonal antibody in immunoadsorption chromatography, eluting IFN-α from the column and keeping it at low pH at least for 16 h, neutralizing the eluate and separating residues of the mouse antibodies by gel filtration and finally subjecting the purified drug substance thus obtained to virus filtration and sterile filtration in a formulation suitable for the finished product. By using at least two monoclonal antibodies with different, preferably complementary specificities, it is possible

to obtain a multicomponent α-interferon preparation containing all the major α–interferon subtypes.

Similar processing steps are used for isolating interferon from human lymphoblastoid cell or transgenic animal cell cultures or from crude preparations obtained from transgenic animals.

Viral safety of the product is confirmed by providing at least two effective virus elimination steps for all viruses representing diverse physicochemical properties. First, S/D treatment inactivates effectively all enveloped viruses. Second, immunoadsorption chromatography with the excessive washing steps removes all types of viruses. Third, incubation at low pH effectively inactivates most viruses, except some acid-resistant viruses. Finally, virus filtration removes all viruses, including even the smallest non-enveloped viruses. Furthermore, other physicochemically resistant infectious agents, such as "prions", are also effectively removed in the immunoadsorption chromatography and virus filtration steps. Thus, the process comprises at least two effective steps against all types of physicochemically resistant infectious agents, and even four steps for the most important blood-derived viruses, HI-viruses and hepatitis B and C viruses, which carry a lipid envelope and are effectively inactivated in S/D and low pH treatments and removed in immunoadsorption chromatography and virus filtration.

An exemplifying embodiment of the manufacturing process is depicted in Figure 1, which illustrates the process starting from a leukocyte culture.

Referring to Figure 1, it can be noted that the first step of the preferred embodiment comprises producing a crude interferon in leukocyte culture, in particular by Sendai virus induction (Cantell et al., Methods Enzymol. 78, 29-38, 1981). However, the starting material can equally well be crude interferon obtained in human lymphoblastoid cell culture by Sendai virus induction (Mizrahi et al., Methods Enzymol. 78, 54-66, 1981).

The cells are removed from the culture by decantation, microfiltration and/or by centrifugation. The filtrate or supernatant is concentrated by ultrafiltration to provide a crude IFN concentrate, filtered and treated with S/D reagents in order to decompose the lipid enveloped viruses. S/D treatments are disclosed in the art, in particular in US Patents Nos. 4,540,573, 4,764,369 and 4,820,805, which are herewith incorporated by reference. The organic solvent is preferably selected from dialkylphosphates and trialkylphosphates

having alkyl groups which contain 1 to 10 carbon atoms. Examples of trialkylphosphates are tri-(n-butyl)phosphate, tri-(t-butyl)phosphate, tri-(n-hexyl)phosphate and tri-(2-ethylhexyl)phosphate. The concentration of the solvent is in the range of 0.001 % to 10 %, preferably about 0.01 % to about 1 %, a typical concentration of tri-(n-butyl)-phosphate being about 0.3 % (calculated from the weight of the solvent). The solvent can be used together with a non-toxic detergent, which is capable of enhancing the effectiveness of the solvent. Such substances are exemplified by non-ionic surfactants, e.g. oxyethylated alkyl phenols, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene alcohols and sodium deoxycholate. The amount of the detergent is in the range of 0.001 to 10 %, preferably about 0.01 to 1.5 %. Typically, the detergent comprises 1 % polysorbate 80 (Tween 80), 1 % Triton X-100 or 0.2 % sodium deoxycholate. S/D substances may be used even at higher concentrations, such as 2 % tri-(n-butyl)phosphate without a detergent. The treatment time may be 4-50 h, and the temperature 24-30 °C.

Protein impurities, DNA, possible residual viruses and S/D chemicals are removed by binding IFN-α to an immunoadsorbent consisting of monoclonal antibodies coupled to a solid matrix. To obtain all major IFN-α subtypes at least two monoclonal antibodies with complementary subtype specificities should be used. It is possible to implement the invention using only one monoclonal antibody.

Monoclonal antibodies can be produced by using purified leukocyte interferon as an immunogen. The antibody producing cell clones are selected by testing the ability of the antibodies to bind leukocyte interferon in immunoadsorption (Example 1). The monoclonal antibodies should together bind at least 90 % of interferon activity present in crude interferon, and the yield of interferon activity eluted with low pH should be at least 80 % of the bound activity. The antibodies should have complementary binding specificities to IFN-α subtypes so that they bind together all major IFN-α subtypes. Particularly, the antibodies should bind at least the IFN-α subtypes α1, α2, α8, α10, α14, α17, and α21. It is preferred to have at least one antibody which binds subtype α1 and one which binds subtype α2.

The immunoadsorbent column is washed extensively and the bound IFN-α is eluted with low pH buffer (pH 2 to 2.5). The elution buffer may contain albumin or other stabilizers, such as a non-ionic detergent, to stabilize IFN-α. The eluate is incubated at low pH at least for 16 h to inactivate possible residual viruses. The eluate is neutralized by adding alkaline buffer or sodium hydroxide. The neutralized eluate is concentrated by ultrafiltration.

Alternatively, IFN-α can be bound to an ion-exchange chromatography gel.

Mouse IgG and its fragments leaking from the immunoadsorbent and possible albumin stabilizer are removed by gel filtration or ion-exhange chromatography. The IFN-α containing fractions are pooled and the purified IFN-α drug substance thus obtained is stored frozen at -70 °C.

The IFN-α drug substance is stabilized with a suitable stabilizer, such as albumin or a non-ionic detergent, and filtered with a virus removal filter with high retentive capacity even for the smallest non-enveloped viruses. In the present context, virus filters capable of reducing the concentration of model viruses, preferably model viruses having a size of 20 to 40 nm, in the spiking tests with at least 4 log, are considered to have a "high virus retentive capacity". It is particularly important that the filters used have such capacity also in relation to small non-enveloped viruses. Based on the spiking tests with model viruses, the theoretical pore sizes of the virus removal filters can be set at about 10 to 40 nm, preferably about 10 to 20 nm.

The yield of IFN-α in virus filtration and the capacity of the filter are clearly higher when a non-ionic detergent, such as polysorbate 80, is used as a stabilizer instead of albumin, as indicated in Example 4. Two virus removal filters can be used sequentially, which further improves virus retentive capacity. Virus filtration may be also performed after sterile filtration of the formulated bulk solution. Additional stabilizers may be added to the bulk solution after virus filtration.

It is also possible to formulate the IFN-α drug substance to a suitable pharmaceutical composition before the virus filtration step, as indicated in Figure 1.

The process according to the present invention effectively removes all types of impurities present in leukocyte and lymphoblastoid cell cultures, as indicated by more than million fold removal of DNA and about million fold removal of ovalbumin, which is the major heterologous protein derived from Sendai virus preparation used in the induction of IFN-α production in the cells (Example 2). The level of mouse IgG leaking from the immuno-adsorbent is typically below 10 ng/3 mill. IU of purified IFN-α.

The present process is economical containing only two chromatography steps, and it gives good yield of the purified drug substance, on an average about 60 % (calculated from the

immunochemical concentration of IFN-α) as shown in Example 2.

By the present process it is possible to obtain IFN-α with consistent subtype composition, which has been demonstrated in Example 3 by comparing the subtype composition of 13 different drug substance batches. The use of monoclonal antibodies originating from different antibody production batches results in a very similar IFN-α subtype composition, which illustrates the capacity of the process to yield a product with constant quality in long term production.

The following non-limiting Examples illustrate the invention:

Analytical Methods used in the Examples

IFN-α concentration

The IFN-α concentration was measured by a time-resolved fluoroimmunoassay (FIA) on microtitre plates. The IgG fraction of a bovine antiserum against human leukocyte IFN-α was used in capturing and a mixture of two Eu-labelled mouse IgG monoclonal antibodies to IFN-α for detection. The monoclonal antibodies were the same as used in the purification of IFN-α. The details of the assay have been described elsewhere (Rönnblom et al., APMIS 105, 531-536, 1997). IFN-α concentration was expressed as IU/ml using a laboratory standard, which was calibrated by the virus plaque reduction assay against the International Reference Preparation of Interferon, Human Leukocyte 69/19 (NIBCS, U.K.).

Interferon antiviral activity

The antiviral activity of the IFN was determined by a virus plaque reduction assay in 35 mm petri dishes using Human Epithelial 2 (HEp2) cells challenged with Vesicular stomatitis virus (VSV). The IFN-α samples, control and standard were diluted serially at 0.25 log intervals to concentration of 0.3 - 3 IU/ml in Eagle's Minimum Essential Medium (EMEM) supplemented with fetal calf serum (FCS) 7 % and aureomycin 0.004 %. The samples were assayed as triplicates at four dilutions in at least two assay series. One ml of cell suspension (2 x 10^6 cells/ml) in EMEM and 1 ml of sample dilution were added to dishes. Virus control dishes without IFN were included in each assay series. After incubation of overnight at 37 °C in 3 - 4 % CO_2 atmosphere the solutions were removed from the confluent cell layers and 150 - 200 PFU of VSV in 1 ml of EMEM was added. After incubation of 40 - 45 min the virus was removed and cells were overlayed with 2 ml of agar 0.8 % in EMEM. After overnight incubation the virus plaques were calculated.

One unit of IFN activity is the highest dilution of the sample, which inhibits 50 % of the virus plaques as compared to the virus control. Interferon activity was expressed in International Units (IU) using a laboratory standard, which was calibrated against the International Reference Preparation of Interferon, Human Leukocyte 69/19 (NIBCS, UK).

Total protein

Total protein concentration was measured according to Lowry using human albumin as a standard (Total Protein Standard, Finnish Red Cross Blood Transfusion Service, Helsinki, Finland).

Mouse IgG

Mouse IgG concentration was measured by enzyme immunoassay (EIA) on microtiter plates. Polyclonal goat anti-mouse IgG_1 (Fc) $F(ab')_2$ fragment (Jackson Immuno Research Laboratories, U.S.A.) was used in capturing and polyclonal peroxidase-conjugated goat anti-mouse IgG (H+L) (Jackson Immuno Research Laboratories, U.S.A.) for detection. Mouse myeloma IgG_1 (Cappel, Belgium) was used as a standard.

Ovalbumin

Ovalbumin concentration was measured by a time-resolved fluoroimmunoassay (FIA) in microtitre plates. Polyclonal rabbit IgG against chicken egg albumin (Organon Teknika Co, U.S.A) was used in capturing and polyclonal Eu-labelled goat IgG against chicken egg albumin for detecting (Organon Teknika Co, U.S.A). Chicken egg albumin (Sigma, U.S.A.) was used as a standard. The fluorescence was measured with a time-resolved fluorometer (1234 Delfia Research Fluorometer, Wallac, Finland).

Total DNA

The samples were treated with sodium dodecyl sulphate and proteinase K at pH 11 and the concentration of DNA was measured by the Threshold Total DNA Kit (Molecular Devices, U.S.A.).

IFN-α subtype composition

The IFN-α subtypes were separated by reverse phase high performance liquid chromatography (RP-HPLC). IFN-α sample (c. 1 mill. IU in 400 µl) was applied to a Delta-Pak™ HP1 C4 column (Waters, U.S.A.) and eluted with a linear gradient of 40 - 55 % acetonitrile in 0.1% (*v/v*) trifluoroacetic acid in 57 min with a flow rate of 0.1 ml/min. Absorbance was monitored at 214 nm. IFN-α samples containing human albumin were

first run through a Superdex 75 HR 10/30 column (Pharmacia Biotech, Uppsala, Sweden) in 50 mmol/l Tris buffer, pH 7.5, containing 0.15 mol/l NaCl to remove albumin from IFN-α.

For the identification of IFN-α subtypes in the RP-HPLC peaks the fractions were subjected to N-terminal sequencing and peptide mass mapping either directly or after further separation by SDS-PAGE following procedures described in detail elsewhere (Nyman et al., Biochem. J. 329, 295-302, 1998).

Examples

Example 1
Production of monoclonal antibodies against IFN-α

This example describes the production and selection criteria for the monoclonal antibodies which are used in the immunoadsorption step of the described process. IFN-α used for immunization was partially purified leukocyte interferon (Finnferon-alpha, Finnish Red Cross Blood Transfusion Service), which had been further purified by immunoadsorption using bovine polyclonal anti-IFN-α antibodies coupled to CNBr-Sepharose (Pharmacia Biotech, Sweden). Bound IFN-α was eluted at low pH and stabilized with sodium dodecyl sulphate (SDS). The immunization procedure consisted of giving one intraperitoneal injection of 5 mill. IU of IFN-α in 0.25 ml complete Freund's adjuvants followed by 4 injections of 5-10 mill. IU without adjuvant with c. 14 days intervals to female Balb/c mice. Spleen cells from immunized mice were prepared for fusion 4 days after the last injection.The fusion partner cell line was a non-secreting mouse myeloma cell line Sp2/0-Ag14 (ATCC CRL 1581), and thymocytes prepared from Lewis rats were used as feeder cells. Fusing agent was polyethylene glycol 1500 and the medium was DMEM supplemented with HT and 10 % FCS.

After fusion, cells were subcloned and tested for the production of mouse monoclonal antibodies against human IFN-α in enzyme immuno assay using the purified human leukocyte IFN-α as an antigen. In the next screening step, strongly positive monoclonal antibodies were selected for further testing. In the principal screening for binding, the monoclonal antibodies were incubated with human leukocyte interferon. Rabbit anti-mouse IgG antibodies coupled to Sepharose 4B gel (Pharmacia Biotech) were added and after incubation, the gel with bound mouse monoclonal antibodies and IFN-α was

separated by centrifugation. The supernatant was determined for interferon activity. Antibodies which bound at least 70 % of interferon activity were selected for further testing in combination of two different antibodies, and antibodies which as a mixture bound at least 90 % of the activity were considered as suitable candidates for the immunoadsorption chromatography step. Further testing was carried out by coupling the monoclonal antibodies to CNBr-Sepharose 6B (Pharmacia Biotech) and testing their binding properties in immunoadsorption. The selection criterium was that two monoclonal antibodies should together bind at least 90 % of interferon activity of crude leukocyte interferon and that the yield of the activity eluted with low pH should be at least 80 % of the bound activity. The two mouse IgG monoclonal antibodies selected for the purification process bind together all major leukocyte IFN-α subtypes (IFN-α1, IFN-α2, IFN-α8, IFN-α10, IFN-α14, IFN-α17, and IFN-α21). Neither of them binds beta- or omega-interferon. A typical RP-HPLC chromatogram of the product purified by using these two monoclonal antibodies in immunoadsorption is shown in Figure 2.

Example 2

Purification of IFN-α drug substance from crude interferon

This example was performed by following the steps from crude interferon to purified drug substance in the scheme of Figure 1. The cells were removed from the leukocyte culture first by decanting and by microfiltration using 0.3 μm membranes. The filtrate was concentrated 20-fold by ultrafiltration using 10 kD membranes. If not used immediately the concentrate was stored frozen at -40 °C. After thawing, the concentrate was filtered through 1.2 μm and 0.22 μm filters, and the clarified concentrate was treated with 0.3 % tri(n-butyl)phosphate and 1 % polysorbate 80 for 16 h at 26 °C.

After S/D treatment, the concentrate was applied to an immunoadsorbent column containing the two monoclonal antibodies coupled (4 mg/ml of each) to CNBr-Sepharose 4FF gel (Pharmacia Biotech). The load was 30-40 mill. IU IFN-α per ml gel. The immunoadsorbent column was washed with 40 column volumes of 0.011 mol/l sodium phosphate buffer, pH 7, 0.14 mol/l NaCl (PBS) containing 0.1 % polysorbate 80 followed by 10 column volumes of PBS without polysorbate 80. The bound IFN-α was eluted with pH 2-buffer. Human albumin 0.2 g/l (Finnish Red Cross Blood Transfusion Service) was added to pH 2-buffer to stabilize IFN-α. After incubation for 18 - 20 h at pH 2, the eluate was neutralized by adding slowly 0.5 mol/l NaOH and concentrated about 30-fold by ultrafiltration using 10 kD membranes.

The concentrated eluate was applied to a Superdex 75 column (Pharmacia Biotech) equlibrated with PBS. The IFN-α containing fractions were pooled and stored frozen at -70 °C. The yield of IFN-α in 11 purification batches is shown in Table 1.

Table 1. Yield of IFN-α in the purification process; mean values calculated from IFN-α FIA results of 11 purification batches are shown

Process step	Yield per step %	Cumulative yield %
Crude interferon	100	100
Crude interferon concentrate	95	95
S/D-treated concentrate	98	93
Immunoadsorption eluate	80	74
Neutralized and concentrated eluate	89	66
Purified drug substance	91	60

No other protein constituents besides the IFN-α subtypes can be detected in the purified drug substance by silver staining of SDS-PAGE gels. When albumin is used in the immunoadsorption step as a stabilizer, the purified drug substance may contain albumin up to 20 % of total protein. Sensitive immunochemical assays have been used for the measurement of main heterologous protein impurities which are ovalbumin and mouse IgG. The amount of ovalbumin per a standard dose of 3 mill. IU IFN-α has been 0.1-0.3 ng and that of mouse IgG 1-9 ng in the drug substance batches. The purification of IFN-α has been c. million fold in relation to ovalbumin (Table 2).

The amount of the main process-derived impurity, tri(*n*-butyl)phosphate, has been less than 0.2 µg per 3 mill. IU of IFN-α in three drug substance batches analyzed. The amount of DNA was measured in two drug substance batches and found to be less than 60 pg per 3 mill. IU of IFN-α. The purification of IFN-α was more than million fold in relation to DNA (Table 2).

Table 2. Purification of IFN-α in relation to ovalbumin and DNA in the purification process

Process step	Ovalbumin ng/3 mill. IU IFN-α	Purification of IFN-α in relation to ovalbumin	DNA pg/3 mill. IU IFN-α	Purification of IFN-α in relation to DNA
Crude IFN concentrate	1.8×10^5		1.1×10^8	
Purified drug substance	0.2	0.9×10^6	< 60	$> 2 \times 10^6$

Example 3

IFN-α subtype composition in different drug substance batches

Figure 2 shows a typical RP-HPLC chromatogram of the purified drug substance obtained by using the process described in Example 2. The RP-HPLC chromatogram was devided into seven peak groups as indicated by vertical lines in Figure 2. The comparison of 11 drug substance batches indicates that the relative peak areas and thus the subtype composition remained very consistent in different batches (Table 3).

Table 3. IFN-α subtype composition in 11 drug substance batches. The proportion of the different subtypes analyzed by RP-HPLC is shown

RP-HPLC peak group	IFN-α subtype	Average (%)	RSD (%)	Range (%)
1	IFN-α14	7.2	15.8	6 - 10
2	IFN-α2	19.7	4.7	18 - 22
3	IFN-α21, incl. IFN-α4	9.9	12.4	8 - 11
4	IFN-α10	8.7	7.7	7 - 10
5	IFN-α17, incl. IFN-α7	15.6	10.9	13 - 18
6	IFN-α8	6.7	13.1	5 - 8
7	IFN-α1	32.0	7.8	28 - 35

When different production batches of the two monoclonal antibodies were used in the

preparation of the immunoadsorbent, a very similar IFN-α subtype composition was obtained with both immunoadsorbents (Table 4).

Table 4. IFN-α subtype composition in drug substance batches purified by immunoadsorbents prepared of monoclonal antibodies obtained from two different antibody production batches

RP-HPLC Peak group	IFN-α batches (n=2) purified with antibodies from first batches Relative peak area (%)	IFN-α batches (n=11) purified with antibodies from second batches Relative peak area (%)
1	7.9	7.2
2	20.6	19.8
3	10.7	9.9
4	8.2	8.7
5	14.2	15.7
6	6.1	6.7
7	32.4	32.0

Example 4

Manufacture of purified IFN-α finished product

This example was performed by formulating the purified drug substance and subjecting it to virus filtration and sterile filtration according to the scheme in Figure 1. The purified IFN-α drug substance was diluted to concentration of c. 4 mill. IU/ml with PBS containing 1 mg/ml albumin. The formulated solution was prefiltered through 0.1 μm particle filter and thereafter with Planova 15N filter (Asahi Chemical Industry Co, Japan). The Planova filtration was carried out at room temperature using constant pressure (0.9 bar). The IFN-α yield in the Planova filtration was c. 90 %.

A better yield of IFN-α (c. 100 %) and several fold higher filter capacity was obtained in the virus filtration step, when albumin was replaced with a non-ionic detergent, such as 0.2 g/l polysorbate 80, in the formulation of the purified drug substance.

The virus filtered IFN-α solution was filtered with a 0.1 or 0.22 μm sterile filter and

filled aseptically in the final containers.

Claims

1. A method of manufacturing a highly purified and virus-safe α-interferon product from a crude interferon preparation, comprising

a) subjecting the crude interferon preparation to a solvent/detergent treatment;

b) contacting the solvent/detergent treated composition with monoclonal mouse IgG antibodies in an immunoadsorption step for purifying essentially all or selected α-interferon subtypes;

c) eluting the α-interferon subtypes bound by the monoclonal antibodies;

d) removing residual mouse IgG and other impurities from the eluate to produce a purified α-interferon solution;

e) stabilizing the solution and filtering it with a virus removal filter; and

f) adjusting the final composition of the product for aseptic filling.

2. The method according to claim 1, wherein the solvent reagent is selected from the group of di- and tri-(alkyl)phosphates and optionally used together with a non-ionic detergent.

3. The method according to claim 1 or 2, wherein the crude interferon preparation subjected to the solvent/detergent treatment comprises a supernatant obtained from a human or animal cell culture or a preparation from transgenic animals.

4. The method according to any of claims 1 to 3, wherein the crude interferon preparation subjected to the solvent/detergent treatment comprises a supernatant obtained from a leukocyte or lymphoblastoid cell culture, which has been concentrated.

5. The method according to any of claims 1 to 4, wherein in step b the IFN-α is bound to an immunoadsorbent consisting of at least two monoclonal antibodies with complementary subtype specificities coupled to a solid matrix.

6. The method according to claim 4, wherein the immunoadsorbent binds at least 90 % of interferon activity of the crude interferon preparations and the yield of the activity eluted with low pH is at least 80 % of the bound activity.

7. The method according to claim 6, wherein the monoclonal antibodies are capable of binding IFN-α subtypes α1, α2, α8, α10, α14, α17 and α21.

8. The method according to any of the preceding claims, wherein subsequent to step b, the immunoadsorbent column is washed extensively and the bound α-interferon is eluted with a low pH buffer having a pH of less than 3.0.

9. The method according to claim 8, wherein the elution buffer used contains a stabilizer for α-interferon.

10. The method according to any of the preceding claims, wherein the eluate is incubated at low pH for 1 - 70 h.

11. The method according to claim 8, wherein the eluate is neutralized and concentrated 20- to 40-fold.

12. The method according to any of the preceding claims, wherein step d is carried out by gel filtration or ion-exhange chromatography.

13. The method according to claim 12, wherein the α-interferon-containing fractions are pooled and the pure α-interferon drug substance thus obtained is stored frozen at −70 °C.

14. The method according to any of the preceding claims, wherein α-interferon drug substance is formulated to a suitable pharmaceutical composition and filtered by a virus removal filter with a pore size of 10-20 nm to remove small non-enveloped viruses and other infectious agents that have not been inactivated in the solvent/detergent and low pH treatments or removed in the immunoadsorption chromatography.

15. The method according to any of the preceding claims, wherein, starting from cultured cells, the process comprises in combination the steps of

- removing the cells from the cell culture,
- concentrating the crude interferon thus obtained,
- subjecting it to a solvent/detergent treatment
- adsorbing α-interferons with at least two monoclonal antibodies having complementary subgroup specificities using immunoadsorption chromatography,
- subjecting the eluate of the immunoadsorption chromatography to treatment at a pH of 2 - 2.5,
- neutralizing and concentrating the eluate,
- separating residues of the antibodies by gel filtration,

- adding stabilizer to the drug substance thus obtained and subjecting it to virus filtration, and
- adjusting the final composition of the product and subjecting it to sterile filtration and aseptic filling.

16. The method according to claim 15, wherein a leukocyte culture is used as a starting material.

MANUFACTURING PROCESS

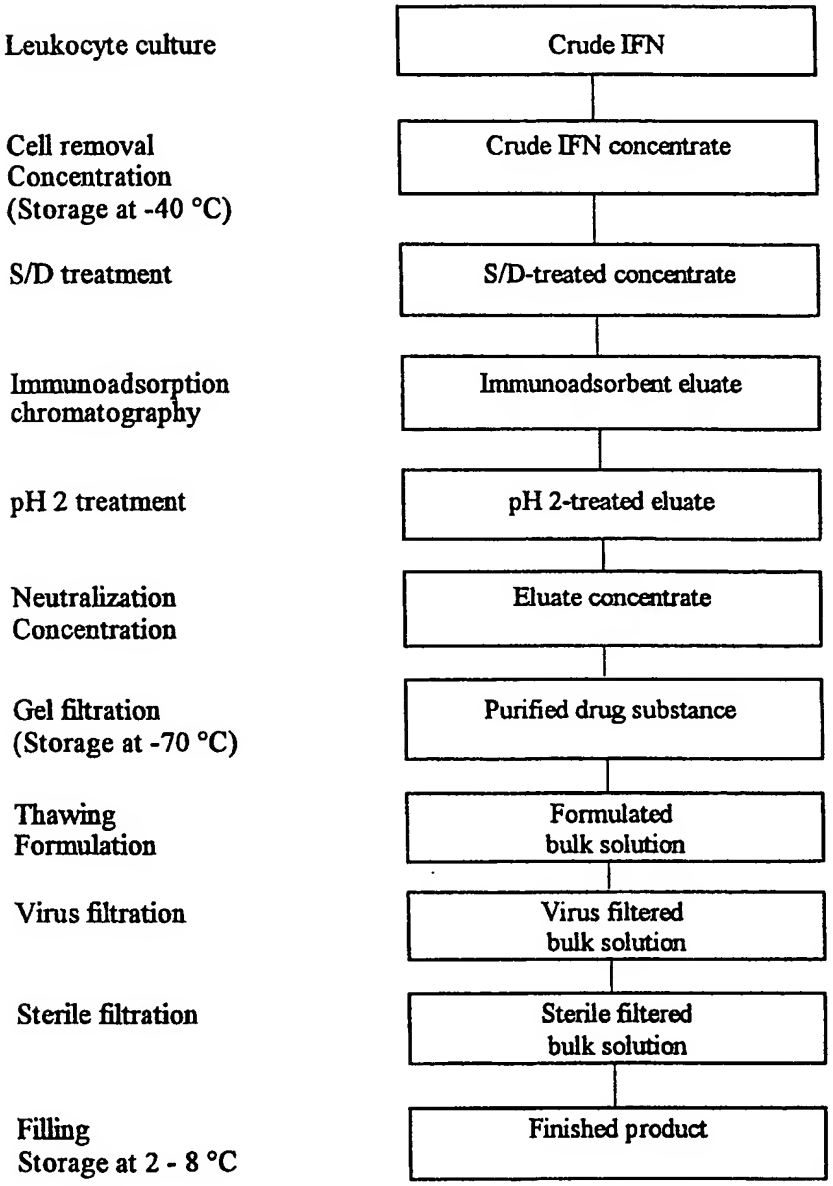

FIG. 1

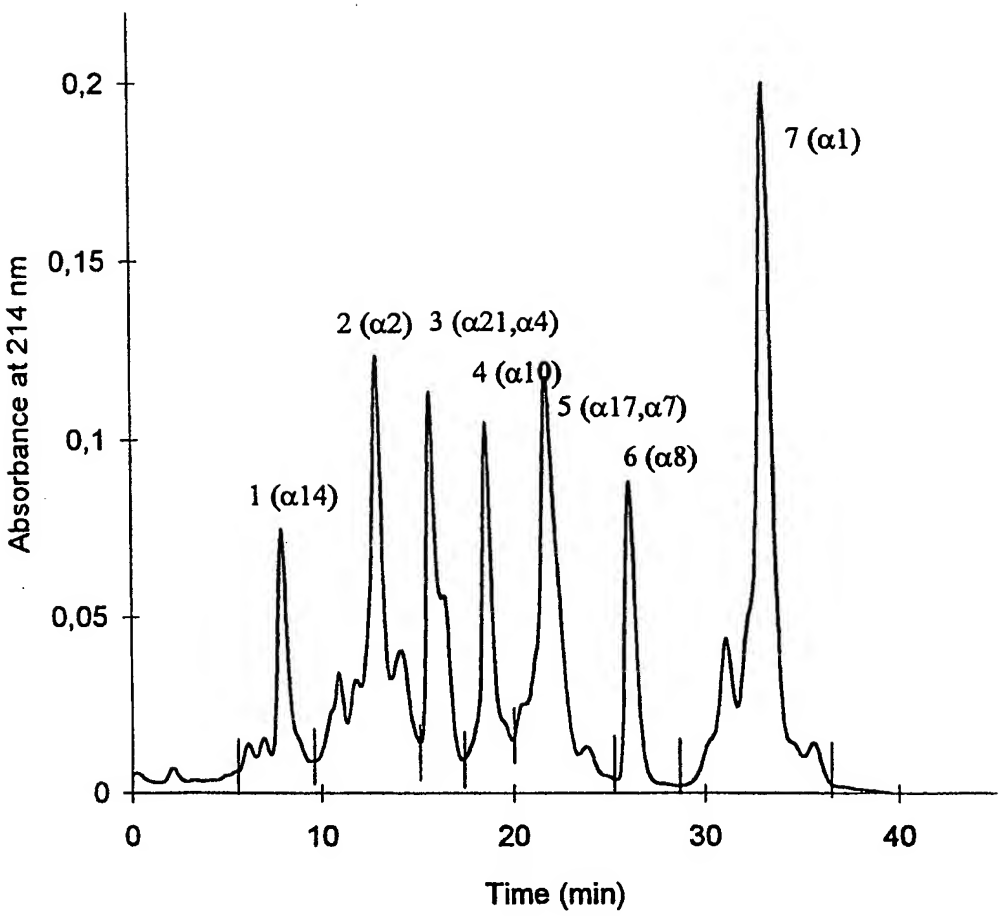
0,2
0,15
0,1
0,05
0
Absorbance at 214 nm
0
10
20
30
40
Time (min)
1 (α14)
2 (α2)
3 (α21,α4)
4 (α10)
5 (α17,α7)
6 (α8)
7 (α1)

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/00506

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 1/22, C07K 14/56, C07K 1/34, A61K 38/21
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 9015817 A1 (BIONATIVE AB), 27 December 1990 (27.12.90), see abstract; page 2, lines 18-26; example 3-9 --	1-14
Y	EP 0152345 A2 (INTERFERON SCIENCES, INC A DELAWARE CORPORATION), 21 August 1985 (21.08.85), see abstract; page 17, line 25 - page 18, line 1; example 2 --	1-14
A	WO 8102899 A1 (SECHER, DAVID, STANLEY), 15 October 1981 (15.10.81), see abstract --	1-16

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
8 Sept 1999	15 -09- 1999
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Carl-Olof Gustafsson/EÖ Telephone No. +46 8 782 25 00

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INTERNATIONAL SEARCH REPORT

Information on patent family members

02/08/99

International application No.
PCT/FI 99/00506

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